Section 1: Introduction

Hemoglobin biology and the functional importance of the oxyhemoglobin dissociation curve

Hemoglobin is crucial to the transportation of oxygen within the circulation of all vertebrates, and some invertebrates. The evolution of globin genes over time has led to the complex structure we are familiar with in human blood today. Hemoglobin is a heterotetramer consisting of two alpha and two beta polypeptide chains, each with a central heme group comprised of an iron ion and a porphyrin ring.(1, 2) The iron-containing heme group on each of the four protein subunits permits binding of one oxygen molecule. Oxygen is bound to hemoglobin in regions of high partial pressure of oxygen (pO_2), such as the lungs, and then released into the tissues, where pO_2 is low due to cellular oxygen consumption. The pO2 varies between normal tissues and this range can be considered "physioxia". (3) The affinity of hemoglobin for oxygen in relation to pO_2 is described by the oxygen-hemoglobin dissociation curve (ODC) illustrated in Figure 1. One of the unique features of hemoglobin is that it exhibits molecular cooperativity, responsible for the sigmoid shape of the ODC, that enables hemoglobin to alternate between two structures; the relaxed (oxyhemoglobin) and tense (deoxyhemoglobin) states, the latter

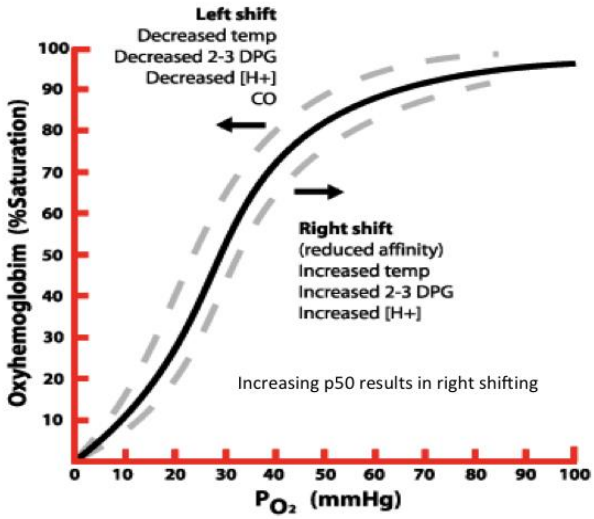


Figure 1: The Oxy-hemoglobin dissociation curve describes the relationship between the partial pressure of oxygen (pO2) and the saturation of hemoglobin with oxygen (% oxyhemoglobin saturation). The p50 is a variable that describes the pO2 at which oxyhemoglobin is 50% saturated and demonstrates a right shift/lower affinity in conditions where tissues require more oxygen delivery or a left shift where affinity is higher and oxygen dissociation from oxyhemoglobin occurs less readily.

demonstrating lower affinity for oxygen. This allows flexibility in terms of how much oxygen hemoglobin can bind, ensuring that the functional groups attain maximal affinity for oxygen. When a monomer of hemoglobin binds a molecule of oxygen a conformational change is induced in the three neighboring monomers, increasing their affinity for oxygen. Cooperativity also enables flexibility in how much oxygen is released to tissues. The p50 of hemoglobin is the pO2 at which hemoglobin is 50% saturated with oxygen (50% oxyhemoglobin, 50% deoxyhemoglobin), and quantifies the affinity of hemoglobin for permitting comparison of the relative affinities of different hemoglobins or conditions. The normal p50 range in an adult is 24–28 mmHg (3.2-3.7 kPa), however, the value varies throughout the body depending on the local environment (for example temperature and pH). The standard p50 ($P50_s$) is an idealized value calculated from the measured pO_2 when the temperature of blood is 37.0 ^oC, partial pressure of carbon dioxide (pCO_2) is 40 mmHg, blood pH is 7.40 and carboxyhemoglobin (COHb) is < 2%. In contrast, the in vivo p50 reflects the oxygen tension at which hemoglobin is 50% saturated at the pH, pCO_2, temperature and COHb level of the subject.

To accurately determine the p50, the ODC must be constructed or a Hill plot created.(4) In clinical practice, arterial blood gas machines estimate the p50 using a single-point measurement and hemoglobin-oxygen saturation.(5) Most devices require the oxyhemoglobin saturation to lie on the straight section of the ODC, therefore desaturated, venous blood should be used. Alternatively, the Siggaard-Anderson oxygen status algorithm(6), incorporating the Tahn equation, is the single-point method that can most accurately calculate the p50 up to an oxygen-hemoglobin saturation of 97%.

Principles of oxygen transport and the physiology of oxygen delivery

Uptake of oxygen into the blood from the alveolus is determined by the pO_2 gradient across the alveolar membrane, the properties of the membrane, and the flow of blood through the pulmonary circulation, alongside physical properties the hemoglobin molecule. Oxygen has a low solubility in plasma (0.225ml per kPa of oxygen per liter of blood), therefore without hemoglobin, the circulation would not be able to transport sufficient oxygen to organs. The actual volume of oxygen that can bind to hemoglobin in vivo remains controversial; the theoretical maximum volume of oxygen that can be carried by hemoglobin is 1.39 ml of O_2/gram of Hb(7); however, this figure does not account for variant species of hemoglobin that are unable to bind oxygen, such as carboxyhemoglobin and methemoglobin. Human studies have demonstrated that a value of 1.34 ml of O_2/gram of Hb might be more realistic.(8)

Table 1 Relevant equations

$C_aO_2 = ([Hb] * H * S_aO_2) + (P_aO_2 * a_{O_2})$ and $C_vO_2 = ([Hb] * H * S_vO_2) + (P_vO_2 * a_{O_2})$
$\dot{D}O_2 = \dot{Q} * C_aO_2$
$AVO_2diff = C_aO_2 - C_vO_2 \approx [Hb] * H * (S_aO_2 - S_vO_2)$
$\dot{V}O_2 = \dot{Q} * AVO_2diff$
$O_2ER = {\dot{V}O_2}/{\dot{D}O_2}$

ABBREVIATIONS
[Hb] = hemoglobin concentration (g/dL)
H = Hufner's Constant (1.34 mL O_2/g Hb)
S_aO_2 or S_vO_2 = arterial or venous oxygen saturation percent
a_{O_2} = solubility of oxygen in water = 0.0031 mL O_2/mmHg/dL
P_aO_2 or P_vO_2 = arterial or venous oxygen tension (mmHg)
$\dot{Q}$ = cardiac output (L/min)

The amount of oxygen carried within arterial blood is referred to as arterial oxygen content (CaO_2) (Table 1). Approximately 98% of circulating oxygen is carried bound to hemoglobin whilst the remaining 2% is dissolved in plasma.(9) Systemic oxygen delivery (DO_2) is the total volume of oxygen, per minute, transported to the tissues from the lungs via the circulation. Oxygen delivery is a product of cardiac output and CaO_2 (Table 1). In a healthy male adult of 70kg weight, systemic oxygen delivery is approximately 1000 ml/l/min (the product of 200ml/l CaO_2 and 5 l/min cardiac output).

At the tissue level, oxygen supply and demand is dependent on a number of factors including diffusion limitation of oxygen from the microcirculation to mitochondria and an array of variables impacting on local tissue oxygen consumption (including sedation, tissue edema, hypothermia/cooling and metabolic rate increased with seizures, burns or sepsis). Likewise, oxygen delivery can be optimized by the manipulation of cardiac output, hemoglobin and SaO_2. However, it is the affinity of hemoglobin for oxygen, its modulation and its potential impact on capillary oxygen supply that is the focus of this article.

Physiological modulation of the p50 and its relevance to peripheral oxygen delivery

The p50 is a measure of the affinity of hemoglobin for oxygen that determines the release of oxygen from the microcirculation into the tissues. An increased p50 (equivalent to a rightwards shift of the ODC in Figure 1) is indicative of a decreased hemoglobin-oxygen binding affinity promoting the release of oxygen to the tissues. Such an increase in p50 can be considered advantageous within tissues that have a high metabolic rate, and therefore high oxygen consumption, such as active skeletal muscle.

The relevance of an increased p50 to peripheral oxygen delivery is illustrated by the physiological responses to acute altitude acclimation and anemia, both of which result in a compensatory increase in production of 2,3-DPG in an effort to restore the tissue oxygenation lost with hypoxemia or anemia. With regards to the physiological process of acclimatization to altitude, a study to assess p50 during a simulated ascent of Mount Everest, Operation Everest II,(10) demonstrated an increase in p50 at a simulated altitude of 29,029 ft; most likely as a result of the hypoxia-induced increase in 2,3-DPG(11).

Conversely, a leftward shift of the oxygen dissociation curve (a decrease in p50), results in increased hemoglobin-oxygen affinity and hence decreased oxygen delivery to the peripheral tissues. Tissue oxygen extraction becomes impaired, hence tissue hypoxia may exist despite a normal PaO_2, as reflected in the compensatory erythrocytosis seen in high affinity haemoglobin variants.(12) Fetal hemoglobin (HbF) has a lower p50 than adult hemoglobin (approximately 19 mmHg), an adaptation to the hypoxic conditions *in utero* that promotes superior oxygen affinity, which in turn facilitates oxygen extraction from maternal hemoglobin across the placental membrane. Likewise, methemoglobin (MetHb) and carboxyhemoglobin (COHb) have increased affinity of hemoglobin for oxygen, and will decrease the apparent p50 even if the natural hemoglobin p50 remains the same. Myoglobin has a p50 of approximately 3 mmHg, an extraordinarily high affinity for oxygen, essential for myoglobin's role in storing, loading and unloading oxygen in the PO_2 range of active skeletal muscle.

Section 2:

Haemoglobin variants with an altered p50

In 1961, Reissmann *et al* described a case of familial cyanosis secondary to a hemoglobin variant with abnormally low oxygen affinity.(13) This variant (hemoglobin Kansas) was first identified in a 14-year old with cyanosis since birth. He was entirely asymptomatic aside some periods of weakness after severe exertion, but had markedly diminished oxygen saturation in the presence of normal oxygen partial pressures. An *in vivo* ODC demonstrated a marked shift to the right with an increase in p50 to ~70mmHg.

Similarly, Avellan-Hietanen *et al.* reported a case of a patient presenting with pneumonia, but with an unexpectedly low SpO_2. The low SpO_2 persisted after recovery, despite lack of hypoxic manifestations and normal exercise tolerance, and a rare point mutation in the alpha-chain-coding gene known as hemoglobin Titusville was discovered.(14) This point mutation resulted in a lower oxygen affinity (p50 ~40mmHg), hence the SpO_2 of arterial blood appeared low while the pO_2 was normal.

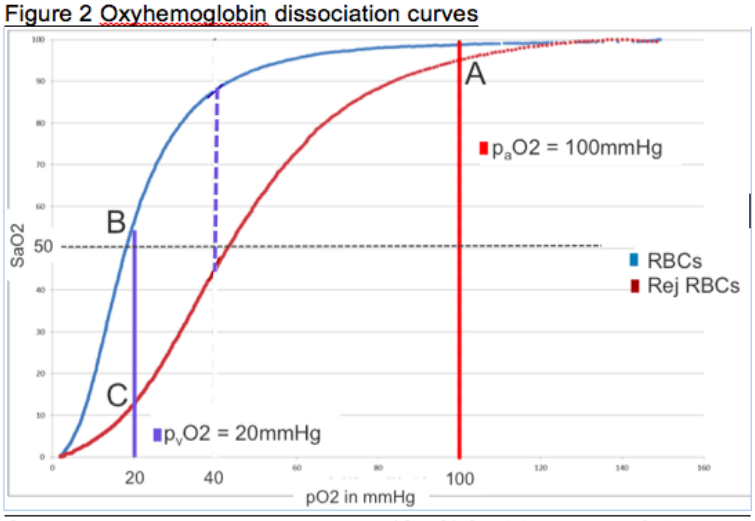
Conversely, high oxygen affinity hemoglobins are responsible for around 100 rare and heterogenous autosomal dominant genetic diseases, resulting in decreased tissue oxygen delivery and erythrocytosis. The diagnosis is based on the identification of a decreased p50 on arterial blood gas analysis and their characterisation by capillary electrophoresis. Many of the mutations associated with a high affinity phenotype map to the $\alpha 1\beta 2$ hemoglobin subunit, preventing the transition of the Hb molecule to the low oxygen affinity state (T or tense) and hence inability to release bound oxygen molecules.(15)

Clinical relevance of p50

Of particular note,is the observation that the p50 of transfused blood is un-

physiologically low; (16) the potential deleterious effects of that is illustrated in Figure 2.

Figure 2 Oxyhemoglobin dissociation curves



Oxyhemoglobin dissociation curves (ODC) for 21 day-old AS-1 blood before (blue) and after rejuvenation (red). A denotes the arterial point, B and C estimate the venous saturations in exercising muscle.

The blue ODC represents 21 day old PRBCs with an SaO2 of 99% and the lowest achievable SvO2 is 54%, assuming an arterial pO2 of 100mmHg and the venous pO2 in exercising muscle of 20mmHg.(17) If the p50 of this unit is allosterically modified by increasing 2,3DPG levels to 150% normal (the right-shift, red ODC), the SaO2 is 95% and the lowest achievable SvO2 is 12%. The A-V difference, therefore, is 83% for high p50 blood and 45% for low p50 blood, a huge difference. Considering that VO_2 is a product of cardiac output and (SaO2-SvO2), each unit of high p50 gives almost twice as much VO_2 increase as a low p50 unit, assuming the cardiac output remains constant. Transfusing blood with an increased oxygen affinity, such that it is less likely to off-load oxygen in the tissues, seems counter-intuitive. However, excessively elevating the p50 into the range seen with the Hb mutants, Titusville or, especially, Kansas, may worsen hypoxemia in the setting of acute lung injury. Several questions remain unanswered regarding the optimal p50 target. Nevertheless, the manipulation of oxygen affinity and hence development of a future therapeutic target to increases VO_2 ideally with lower cardiac output requirements or lower transfusion thresholds, has exciting potential.

Relationship between p50 and VO_2

The major determinant of exercise capacity is the amount of oxygen available to exercising muscles, a function of DO_2 and the extraction ratio that determines achievable VO_2. The upper limit of oxygen utilization during exercise (VO_2max) is defined as the point at which progressively increasing work rate fails to elicit further increases in oxygen uptake. VO_2max coincides with the point of physical exhaustion(18) and is the most commonly used, and highly reproducible,(19, 20) measure of aerobic fitness. While used as a measure of fitness, VO_2 max also reflects the maximum achievable VO_2 in the setting of acute illness and offers insight into how we can manipulate this important parameter to optimize hemodynamics, avoiding shock and lactic acidosis. In health, arterial hemoglobin is close to fully saturated so increasing VO_2 relies mostly on increasing hemoglobin concentration, raising cardiac output, or by decreasing the oxygen binding affinity of hemoglobin (increasing the p50), such that more oxygen is available and released to the hypoxic tissues, as illustrated by the VO_2 equation in Table 1.(21) Figure 3 illustrates the profound effect of p50 on VO_2max, permitting the same VO_2max to be achieved with a lower Hb if the

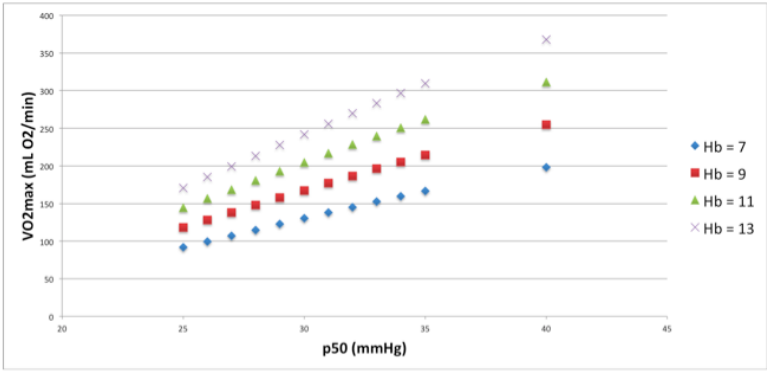


Figure 3: This calculation of VO_2max uses A-V differences based on SaO2 and SvO2 calculated by the Hill equation for blood of varying p50s (x-axis). It shows how increasing the p50 will increase your VO_2max for any level of hemoglobin. Of note with a Hb of 7g/dl and a p50 of 35mmHg, VO_2max exceeds that with a Hb of 11g/dl and a p50 of 25mmHg.

p50 is higher. Similarly, this principle will allow the same VO_2max to be achieved for a lower cardiac output, which may be of benefit for patients with limited cardiac reserve. The data in Figure 4 are the result of a mathematical model of postoperative anemia treated with sequential transfusions of low p50 PRBCs or high p50 PRBCs, similar to those depicted in Figure 2, using the ODCs from Figure 2 to calculate the AVO_2diff (see Table 1 for derivation). As a patient's anemia becomes more profound, they require greater cardiac output to maintain their baseline VO_2. If we consider the pre-operative VO_2, calculated for a cardiac output of 5L/min and a hemoglobin level of 14mg/dL, to be the baseline against which the post-transfusion models are compared, the figure shows the requisite cardiac output necessary to maintain the pre-op VO_2. It is evident that the use of high p50 PRBCs creates a larger AVO_2diff, which permits the CO component of VO_2 to be lower while maintaining the pre-operative VO_2. This represents an important reduction in compensatory cardiac demand required to maintain a given VO_2, which offers benefit in patients with cardiac disease. Similarly, a patient could maintain the same VO_2 with a lower hemoglobin level, potentially reducing the transfusion requirement, as illustrated in Figure 3. It is possible to manipulate the p50 of circulating blood or transfused blood, as outlined in the following sections.

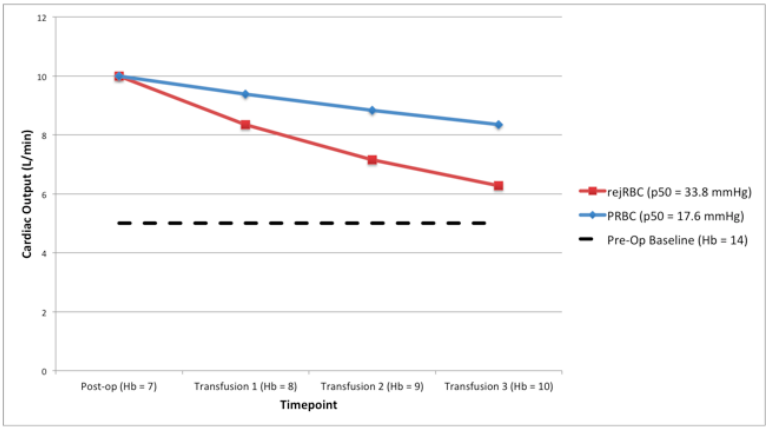


Figure 4: This mathematical model calculates the cardiac output required to maintain a baseline VO_2 following intraoperative blood loss that reduces Hb from 14 to 7 g/dl. It uses A-V differences based on SaO2 and SvO2 for high and low p50 hemoglobin ODCs depicted in Figure 2. After 3 units of blood you can maintain baseline VO2 with 2l/min lower cardiac output by using high p50 versus low p50 blood.

Section 3. Artificial Modulation of p50

RSR-13/Efaproxiral

Developed from the cholesterol lowering agents clofibrate and bezafibrate, structural analogs such as RSR-4 and RSR-13 demonstrated predictable p50 increases in a rat model.(22) Furthermore, it was shown that these fibrate analogs increased tissue oxygenation, thereby confirming the viability of increasing end-organ perfusion through changes in hemoglobin oxygen affinity.(23) RSR-13 improved post-injury mechanical function and ATP concentrations in isolated rat heart models of global myocardial ischemia and hyperkalemic arrest(24), and similar results were seen in dogs subjected to iatrogenic LAD occlusion(25) and hypothermic cardiopulmonary bypass,(26) suggesting potential benefits of increasing tissue oxygenation.

While no increase in reactive oxygen species at the tissue level is seen after RSR-13,(27) it was extensively studied as an adjunct to radiation therapy for patients with metastatic cancer to the brain, as there is evidence of an increase in brain tissue oxygen tension following RSR-13 administration.(28, 29). Phase I trials showed that RSR-13 induced a dose-dependent increase in p50 in patients with glioblastoma multiforme (GBM) receiving radiation therapy.(30) Despite these encouraging early results, the follow-up Phase III study of 515 patients with brain metastases found a modest but insignificant increase in survival.(31) While this research demonstrated a proof-of-concept that increasing p50 could increase tissue oxygenation (in this case cancer cells), it did not translate into a clear survival benefit in brain cancer and was pursued no further.

Inositol Polyphosphates (IHP, ITPP)

Shortly after the discovery of 2,3-DPG as an intracellular allosteric regulator of hemoglobin oxygen affinity(32), attention was turned to other organic phosphates that could influence hemoglobin oxygen binding. Inositol hexaphosphate (IHP) was known to

produce a right-shift in avian hemoglobin(33), and it was shown to reproduce that effect in human blood.(34) However, the clinical feasibility of IHP use was limited by its inability to readily cross RBC membranes. Subsequent work led to the synthesis of inositol tripyrophosphate and *myo*-inositol trispyrophosphate (ITPP)(35), which are transported across the membrane of RBCs through an erythrocyte band-3 dependent mechanism.(36) Kieda and colleagues were the first to show the possible effects of p50 increases on the angiogenesis pathway, finding that human endothelial cells cultured under hypoxic conditions had significantly lower expression of HIF-1α, VEGF, and lower observed angiogenesis when exposed to ITPP-laden RBCs compared to standard human RBCs.(37) A subsequent study in chick ova noted a similar reduction in angiogenesis, and impaired growth of human glioma tissue when grafted onto the ovum as part of an experimental model.(38) Later work in rat models of hepatocellular(39) and pancreatic(40) carcinoma, as well as mouse models of primary(41) and metastatic(42) colorectal cancer, all showed decreases in angiogenesis pathway components, tumor size, and improved animal survival with ITPP alone or as an adjunct to chemotherapy. Currently, a Phase Ib/IIa trial is actively recruiting to test ITPP as an adjunct to standard-of-care chemotherapy in patients with non-resectable hepatopancreaticobiliary cancers,(43) although enthusiasm may be tempered by the failure of RSR-13 to improve outcomes despite promising, early observations.

Hemodynamic and Systemic Effects of Modulating p50 in Model Systems

Several studies in model systems evaluated how modulation of hemoglobin p50 can influence cardiovascular system parameters and tissue oxygenation. An early study by Valeri and colleagues, who pioneered stored erythrocyte rejuvenation, looked at isolated

canine hearts that were perfused by human RBCs containing either 80% or 300% of the normal 2,3-DPG concentration. The latter had a significantly higher p50, and produced significantly higher oxygen consumption by the myocardium at a standardized perfusion rate and, crucially, produce less lactate, confirming improved tissue oxygenation.(44) A similar study involving IHP-loaded erythrocytes in isolated rat hearts re-demonstrated the significant increase in oxygen consumption with high-p50 RBC, but further noted a significant decrease in coronary blood flow.(45) This latter point is particularly salient – the greater offloading of oxygen at the myocardium compensated for an overall decrease in perfusion, a concept that can be explained by considering the equation for calculating oxygen consumption (Table 1).

Oxygen consumption (VO_2) describes the amount of oxygen used in aerobic metabolism. If demand exceeds the maximum achievable VO_2 (VO_2max), the body switches to anaerobic metabolism, (46) with a resulting lactic acidosis that portends a poor prognosis in the critically ill. (47) Clinicians boost VO_2, to avoid anaerobic metabolism and lactic acidosis, by using inotropic support to boost cardiac output, with potentially fatal arrhythmias, or by raising Hb, risking transfusion-associated complications. Increasing p50 and oxygen off-loading in the tissues increases the $SaO_2 – SvO_2$ component in the VO_2 equation and potentially offers a novel, third therapeutic option.

The first *in vivo* studies of p50 modulation were accomplished by Teisseire and colleagues using IHP-loaded erythrocytes in piglets. Following isovolumic exchange transfusion, piglets receiving high-p50 blood were noted to have an increased arteriovenous oxygen differential (AVO_2diff), representing increased oxygen offloading. There was an observed negative correlation between p50 and cardiac output, but again the

tissue oxygen consumption was maintained by the increase in end-organ oxygen release.(48) The same group then observed outcomes at later timepoints in another piglet cohort subjected to the same isovolumic exchange transfusion of high-p50 or normal-p50 RBCs. IHP-loaded RBCs were noted to have a lifespan equivalent to that of normal porcine RBCs, and maintained their elevated p50 for over 20 days following transfusion, during which time interval the piglets maintained pre-transfusion oxygen consumption at significantly lower cardiac outputs.(49) A later pilot study looking at piglets placed on hypothermic cardiopulmonary bypass (CPB) primed with either high-p50 or normal-p50 RBCs found that piglets receiving IHP-loaded erythrocytes had improved left ventricular and aortic pressures after CPB, as well as increased oxygen consumption, suggesting a potential cardioprotective use of high-p50 blood during CPB.(50)

Mouse model systems further explore the systemic effects of p50 modulation and potentially physiological benefit. Using donor blood from a mouse model expressing a mutant, low-affinity hemoglobin (Hb-Presbyterian), Huang and colleagues induced endotoxemia in wild-type mice and transfused them with either normal-affinity RBCs, low-affinity RBCs, or saline. After 7 days, survival was significantly higher in the cohort receiving the low-affinity hemoglobin transfusion, and there was significantly lower hepatocyte apoptosis in this population,(51) suggesting benefit from increasing end-organ tissue oxygenation in this model of sepsis with organ dysfunction. In mouse models of heart failure, Watanabe and colleagues used coronary artery ligation to induce heart failure, after which they used one of two methods (bone marrow transplant from Hb-Presbyterian mice or RSR-13 infusion) to increase p50. Compared to animals receiving control bone marrow or placebo, mice with high-p50 blood had significantly higher

exercise capacity independent of any changes in cardiac function.(52) As such, the heart failure persisted (confirmed by histological evidence) but there was robust improvement in functional status. Similarly, in a transgenic mouse model of dilated cardiomyopathy, both intraperitoneal and oral ITPP resulted in dose-dependent and significant increases in maximal exercise capacity.(21) Cabrales *et al*. tested the physiologic effects of a 35% exchange transfusion in animal models using allosterically modified blood with a range of p50s, and found that the maximal tissue pO_2 was produced by blood that had a slightly elevated p50.(53)

Although formal human studies involving p50 modulation and exercise capacity have not yet been performed, both RSR-13 and ITPP have been listed as prohibited substances for professional athletes(54), given the potential for increases in systemic oxygen consumption and the functional advantages thereof.

Section 4. p50 Modulation in Transfusion Medicine

Red Blood Cell Storage and p50

Despite their status as the most commonly transfused blood product, packed red blood cell units (PRBCs) have been shown to undergo major deleterious changes while in storage. Amongst other changes,(55) after 7 days of refrigerated storage, the p50 is significantly reduced from a baseline of 27 to 22 then to a nadir of 18 mmHg after 21 days storage. (16) This is echoed in lowered 2,3 DPG levels in patients receiving allogeneic units rather than cell-saver blood during surgery, (56) and in older studies that observed reduced 2,3-DPG and p50 in a small cohort of adult patients who underwent cardiac surgery with CPB and received a mean of 18 units of stored blood.(57) This finding was

reproduced in pediatric cardiac surgery patients, where it was noted that the age of transfused PRBCs correlated with the decrease in serum 2,3-DPG.(58) In this clinically relevant setting of acute transfusion, we can see that PRBCs will reduce p50, impairing O2 off-loading in the tissues..

How does this relate to recent large, prospective outcome studies comparing "fresh" to "old" blood? While studies in healthy volunteers(59) and anemic outpatients(60) have found no evidence of microcirculatory impairment with older stored units, there is evidence that subjects receiving autologous units have less boost in VO_2max and exercise duration when receiving units stored for 42 days versus 7 days. (61) A small change in VO_2max is a valid physiological outcome but may not translate into adverse clinical outcomes. The ABLE and RECESS trials addressed the question of RBC storage age in critically-ill and cardiac surgery populations, respectively. ABLE was powered to detect a difference in 90-day mortality between treatment groups, and found no significant difference in the same between the control (mean±SD blood age 22.0±8.4 days) and experimental (6.1±4.9 days) groups.(62) Similarly, RECESS found no difference between the control (mean±SD blood age 28.3±6.7 days) and experimental (7.8±4.8 days) groups' 7-day organ failure scores.(63) Similarly, the INFORM trial demonstrated no mortality differences between "old" (mean±SD 23.6±8.9 days, median 23, IQR 16-31) and "fresh" (mean±SD 13.0±7.6 days, median 11, IQR 8-16) blood.(64).Overall, the evidence does not convincingly argue for a change in transfusion practices on the basis of stored RBC age. However, the concept of p50 modulation as a transfusion intervention has not been formally tested by any of the aforementioned studies, because "fresh" blood had been

stored for an average of 6 ± 5 days (ABLE), 8 ± 5 days (RECESS), or 13 ± 8 days (INFORM), and 2,3 DPG levels would already have been substantially reduced.

RBC Rejuvenation

In the 1970s, Valeri and Zaroulis showed that there was a potential clinical benefit to be obtained from the use of a rejuvenation solution containing pyruvate, inosine, glucose, adenine, and phosphate. Outdated RBC units showed evidence of normalization of 2,3-DPG, ATP, and p50 post-rejuvenation, and the authors demonstrated a significant increase in 24-hour post-transfusion recovery when using the rejuvenation process.(65) Other effects on the storage lesion, mostly from ATP restoration, include reduced osmotic and mechanical fragility(66), membrane lipid peroxidation(67), and injurious RBC-endothelial interactions.(68) Nearly expired units could be rejuvenated and frozen, with acceptable 24-hour post-transfusion recovery and hemolysis measurements when thawed months to years later;(69-71) rejuvenation was primarily introduced and FDA approved to extend the storage life of rare phenotype RBC units.

Figures 3 and 4 model the physiological benefits of high p50 blood in terms of maintaining VO2 at a lower cardiac output or at a lower Hb, with obvious potential applications. This could theoretically be achieved with rejuvenated blood that has a p50 of 35-40mmHg. The logistics of achieving this in the clinical setting is under investigation.

Expert Commentary

Oxygen consumption (VO_2) describes the amount of oxygen used in aerobic metabolism. If demand exceeds the maximum achievable VO_2 (VO_2max), the body switches to anaerobic metabolism, with a resulting deleterious lactic acidosis. Clinicians boost VO_2, to avoid anaerobic metabolism and lactic acidosis, by using inotropic support to boost

cardiac output or by raising hemoglobin levels, risking arrhythmias and transfusion-associated complications.

The formula for VO_2 is a multiplication of hemoglobin level, cardiac output, and arteriovenous oxygen differential (SaO2-SvO2). Increasing p50 and oxygen off-loading in the tissues increases the $SaO_2 - SvO_2$ component in the VO_2 equation and offers a therapeutic alternative to iatrogenic manipulation of CO and hemoglobin level. Current clinical studies are either focusing on increasing systemic p50 with the allosteric modifier *myo*-inositol trispyrophosphate (ITPP), to reduce tumor related angiogenesis, or using an FDA-approved solution of pyruvate, phosphate buffers, inosine, glucose and adenine, approved to extend the life of rare phenotype RBCs prior to frozen storage, to increase the p50 of RBCs prior to transfusion.

5-year view

There is a peaking interest in a third therapeutic option for managing borderline shock states in critically ill patients especially when cardiac function is compromised or minimizing transfusion is desirable and adjuncts to cancer therapy have even broader appeal. Challenging existing transfusion triggers could result from using more efficacious RBC transfusions, with positive effects on inventory and donor exposure. Current studies evaluating increased p50 in the setting of chemotherapy(43) and sickle cell disease (NCT02731157) will inform emerging protocols, and using VO_2max to test this concept of "blood transfusion efficacy" could be particularly applicable in this setting.(61) As a readily available parameter on a venous blood gas, increasing, or at least normalizing, p50 could feasibly become a goal of resuscitation.

Key Issues

- Cooperative binding in the 4 subunits of adult hemoglobin gives rise to the sigmoidal shape of the oxygen dissociation curve (ODC) and allows for the calculation of p50, the partial pressure of oxygen at which hemoglobin subunits are 50% saturated.
- Systemic oxygen consumption (VO_2) is a function of cardiac output, blood hemoglobin levels, arterial and venous oxygen saturation, and p50. VO_2 is increased by moderate elevations of p50 due to increased oxygen off-loading in the tissues.
- Endogenous factors influence p50. Acidosis, 2,3-DPG and hypercapnia cause right-shifting of the ODC, while alkalosis, hypothermia, methemoglobinemia, and fetal hemoglobinemia cause left shifting.
- Increasing p50 in animal models has shown to improve systemic hemodynamics, tissue oxygenation, microvascular function, and exercise capacity.
- Stored RBCs suffer a rapid depletion of 2,3-DPG, a resulting decrease in hemoglobin p50 and accumulate other biochemical/structural changes.
- Commercially available processes to replete 2,3-DPG and ATP in stored RBCs, increase p50 and reverse some storage-related structural abnormalities.
- Hemoglobin p50 demonstrates dose-dependent increases *in vivo* in response to inositol polyphosphates, exogenous 2,3 DPG and the fibrate analog efaproxiral.

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